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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

DYMECKI

Appln. No. 08/866,279 Group Art Unit: 1632

Filed: May 30, 1997 Examiner: A.-M. Baker

FOR: USE OF FLP RECOMBINASE IN MICE

* * *

January 14, 1999

RESPONSE

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Responsive to the Office Action mailed September 14, 1998 (Paper No. 4), consideration of the following remarks is respectfully requested.

Reconsideration and allowance are requested.

Claims 1-49 are pending and have been examined on the merits.

The amendments to the claims find support throughout the disclosure as originally filed. Therefore applicant submits that no new matter has been introduced.

A form PTO-948 has not been received by applicant.

35 U.S.C. 112

Claim 4 was rejected under Section 112, first paragraph, because the specification allegedly "does not reasonably

provide enablement for introducing the Flp-recognition sequences in such a way as to generate a mosaic transgenic mouse wherein at least two diploid cells have different number of Flp-recognition sequences." Applicant traverses.

In a mosaic or chimeric transgenic mouse, cells that have undergone site-specific recombination between Flp-recognition sequences may differ in the number of Flp-recognition sequences they contain because each cell may undergo a different number of Flp-mediated recombination events. See page 20 of the specification.

Stochastic, stage-specific, and/or tissue-specific recombination can be exploited to alter the genotype of a subset of cells within a transgenic mouse. In stochastic recombination, only a subset of cells in each tissue of the transgenic mouse may harbor sufficient Flp activity to mediate recombination. The result could be a mosaic transgenic mouse that bears clones of genetically distinct cells. Moreover, such a mosaic transgenic mouse may allow cell populations related by cell lineage to be traced and fate maps to be constructed. See pages 13-14 of the specification.

As an illustration of this embodiment of the invention, a mosaic transgenic mouse may be used to study competition between wild-type and mutant cells during development and growth of the mouse since its tissues can be mosaic for these two cell types. See Dymecki (Proc. Natl. Acad. Sci. USA, 93:6191-6196, 1996) which was previously submitted in applicant's Information Disclosure Statement and Dymecki and

Tomasiewicz (Dev. Biol., 201:57-65, 1998) which is submitted herewith for examples where Flp-mediated recombination resulted in mosaicism within different transgenic mouse tissues as demonstrated by Southern blot hybridization.

Thus, Flp-mediated recombination in which the number of recombination events among cells in the transgenic mouse is different may give rise to at least two diploid cells that contain different numbers of Flp-recognition sequences in their genomes.

Claims 15, 41-42 and 47 were rejected under Section 112, second paragraph, as being allegedly indefinite. Applicant traverses.

On page 3 of the Office Action, it was alleged that the meanings of the terms "essential gene" and "developmental gene" is unclear. Applicant respectfully disagrees because these terms are well defined in genetics.

An essential gene may be required for viability of an individual cell or organism. For example, a gene encoding an enzyme may be required to perform an essential function in a metabolic pathway. Moreover, adding a functional version of an essential gene as a transgene may be used to rescue a mouse homozygous for a lethal mutation. The alternatives presented by the Examiner are not contradictory, and the meaning of "essential gene" is clear and definite.

Similarly, a developmental gene may be required to control differentiation of an individual cell or development of an organism. Developmental genes may be involved in the

complex, three-dimensional organization of a functional adult organism: for example, genes involved in differentiation, morphogenesis, determination, and/or pattern formation (see, for example, Gillen, ed., Molecular Biology of the Gene, 4th edition, Benjamin/Cummings Publishing Company, Menlo Park, 1987; and Wilkins, ed., Genetic Analysis of Animal Development, 2nd edition, Wiley-Liss, New York, 1993). The alternatives presented by the Examiner are not contradictory, and the meaning of "developmental gene" is clear and definite.

For all of the foregoing reasons, applicant respectfully requests withdrawal of the rejections under Section 112.

35 U.S.C. 102

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." M.P.E.P. § 2131 quoting *Verdegaal Bros. v. Union Oil Co. Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The elements must be "arranged as in the claim." *Lindemann Machinenfabrik v. Am. Hoist & Derrick Co.*, 221 USPQ 481, 485 (Fed. Cir. 1984). In contrast, the references cited in the Office Action (Paper No. 4) do not disclose each and every element arranged as in the pending claims.

Claims 1, 2, 4-19, 22-27, 29-36, 41-43, 45 and 48 were rejected under Section 102(b) as being allegedly anticipated by Kilby et al. (1993). Applicant traverses.

On page 5 of the Action, the Examiner noted that "Kilby et al. did not reduce to practice the generation and use of transgenic mice with the FLP recombinase gene and FRT target sequences." Table 1 of the reference shows that Flp-mediated recombination has not been done to the authors' knowledge, even though Flp appears to have been used in more different species than Cre. In the absence of a disclosure that teaches each and every element of the claimed invention (i.e., the claimed transgenic mouse, method, and system) arranged as in the claims, a prima facie case of anticipation has not been made.

Applicant submits that Kilby et al. does not anticipate the claimed invention because this reference does not put the public in possession of the claimed invention.

Claims 1, 2, 4-13, 22-27, 29-33, 41-43, 45 and 48 were rejected under Section 102(b) as being allegedly anticipated by Wigley et al. (1994). Applicant traverses.

On page 7 of the Action, the Examiner noted that "Wigley et al. did not reduce to practice the generation and use of transgenic mice with the FLP recombinase gene and FRT target sequences." In the absence of a disclosure that teaches each and every element of the claimed invention (i.e., the claimed transgenic mouse, method, and system) arranged as in the claims, a prima facie case of anticipation has not been made.

Applicant submits that Wigley et al. does not anticipate the claimed invention because this reference does not put the public in possession of the claimed invention.

The Examiner is requested to consider the arguments made in the next section prior to using Kilby et al. and/or Wigley et al. to make a new rejection under Section 103(a) in a subsequent Office Action.

For all of the foregoing reasons, applicant respectfully requests withdrawal of the rejections under Section 102.

35 U.S.C. 103

Claims 1, 2, 4-13, 15, 22-27, 29-33, 37-43, 45 and 47-48 were rejected under Section 103(a) as being allegedly unpatentable over Lakso et al. (1992), Wigley et al. (1994), Marx (1993), Marshall (1989), and Bieche et al. (1992). Applicant traverses.

Lakso et al. teach the use of Cre recombinase in a transgenic mouse. As acknowledged on page 8 of the Action, while the reference states "it is likely that other recombinases will be useful in directing precise site-specific DNA rearrangements in transgenic animals," the use of Flp recombinase in transgenic mice is not taught. Thus, the Examiner appears to rely on Wigley et al. to suggest the use of Flp recombinase in transgenic mice.

But a careful reading of Wigley et al. shows that they do not suggest the use of Flp recombinase in transgenic mice. The approach suggested on page 586 shows that transgenic mice are to be generated from ES cells that have been modified by Flp-mediated recombination while in culture. The Flp-mediated recombination contemplated by Wigley et al. would occur in

embryonic stem (ES) cells, not in a transgenic mouse as claimed in the present invention. Although contemplated, this proposal was not reduced to practice by Wigley et al.

Moreover, Wigley et al. describe on page 587 two approaches to supply a pulse of Flp activity in ES cells: transfection of the FLP gene under the control of an inducible promoter and transfection of bacterially-produced Flp protein. In either approach, this reference clearly does not teach or suggest that Flp activity be provided from a FLP transgene in a transgenic mouse.

Marx, Marshall, and Bieche et al. are cited for their teaching various oncogenes and tumor suppressor genes. These references do not address the deficiencies discussed above with respect to Lakso et al. and Wigley et al.

Page 9 of the Action alleges that a reasonable expectation of success would have been anticipated because "the Cre-lox system had already been successfully employed to activate an oncogene in a transgenic mouse." It is further alleged that "the FLP recombinase system is analogous to the Cre recombinase system and functions in a manner that is mechanistically identical to the activity of Cre." Applicant submits that results with Cre cannot be so easily analogized to Flp because the two recombinases do not appear to be identical in their enzymatic functions. Rajewsky's group taught that Flp was not as efficient as Cre in catalyzing recombination in ES cells (page 1160 of Gu et al., 1993). Sauer (Curr. Opin. Biotech., 5:521-527, 1994) is submitted

herewith for its statement on page 524 that Flp catalyzes excision less efficiently than Cre in ES cells. Barinaga (1994), which was previously submitted in applicant's Information Disclosure Statement, reported on page 28 that Flp got a bad reputation when several groups tried to use it to make knockout mice because they had trouble getting it to work well in ES cells. In contrast, page 43 of the specification discloses that Flp recombinase expressed according to the invention can achieve efficient recombination in ES cells on an extrachromosomal substrate.

Lasko et al. and Orban et al. disclosed a Cre transgenic mouse in 1992. But a Flp transgenic mouse was not described in a scientific publication until 1996 (the Dymecki paper submitted in applicant's Information Disclosure Statement). In a letter by O'Gorman and Wahl submitted herewith (Science, 277:1025, 1997), the only publication cited for Flp-mediated recombination in transgenic mice is Dymecki (1996). Also submitted for the examiner's consideration are recent publications using the transgenic line described in the present application (Meyers et al., Nat. Genet., 18:136-141, 1998; Minichiello et al., Neuron, 21:335-345, 1998; and Dymecki and Tomasiewicz, Dev. Biol., 201:57-65, 1998), another line from the Berns group (Vooijs et al., Oncogene, 17:1-12, 1998), and a list of investigators who have requested and received Flp-transgenic mice from applicant.

The foregoing evidence shows that there was <u>not</u> a reasonable expectation of success before the present invention

was made, there was a long lapse of time (about four years) between the publications disclosing transgenic mice with Cre and then Flp, applicant was the first to put the public in possession of the claimed invention, and only one other Flp transgenic line has been published more than two and one-half years after applicant's publication.

Finally, applicant submits that one of ordinary skill in the art would not have been motivated to combine the cited references. The motivation stated on page 9 of the Action is that the combination would "generate a transgenic mouse useful for the study of neoplastic transformation, *in vivo*." But this merely states the result that applicant has achieved, and the resultant combination is not rendered obvious unless the prior art suggests the desirability of the combination. See M.P.E.P. § 2143.01 citing *In re Mills*, 16 USPQ2d 1430, 1432 (Fed. Cir. 1990). Here, the cited references do not suggest the desirability of the combination and there was no reasonable expectation of success in making the claimed invention.

Claims 3, 21, 28, 44, 46 and 49 were rejected under Section 103(a) as being allegedly unpatentable over Wigley et al. (1994), Panigrahi et al. (1992), O'Gorman et al. (1991), Wahl et al. (1997), Hartley et al. (1980), and Buchholz et al. (1996). Applicant traverses.

Wigley et al. allegedly teach the "potential" use of the Flp recombinase system in transgenic mice. As discussed above, however, Wigley et al. do not suggest the use of Flp in

transgenic mice but in ES cells. Furthermore, this suggestion was not supported by a working example.

Panigrahi et al., O'Gorman et al., Wahl et al., Hartley et al., and Buchholz et al. are cited for their teaching various different sequences for Flp and FRT. These references were not cited to address the deficiencies discussed above with respect to Wigley et al.

Page 11 of the Action alleges that a reasonable expectation of success would have been anticipated because "the FLP recombinase gene and FRT target sequences had already been used successfully in cultured mammalian cells as well as in transgenic *Drosophila* (as described in the discussion of the Kilby et al. reference)." Applicant submits that results in tissue culture or with transgenic *Drosophila* cannot be so easily extrapolated to use of Flp in transgenic mice. The recombinase activities documented in Table 1 of Kilby et al. suggests asking the question of why Flp transgenic mice were not done to the authors' knowledge if these results could be readily applied in another context (i.e., a transgenic mouse).

The Stewart group's determination of the thermostability of Flp and Cre recombinases suggests a possible answer to this question and an explanation for the failures of others to make the claimed invention prior to applicant's success: a much lower temperature optimum for Flp than Cre. The abstract of Buchholz et al. (1996) states, "FLP is more thermolabile, having an optimum near 30°C and little detectable activity above 39°C . . . . Cre is optimally efficient at 37°C and

above." They go on to disclose that the F70L mutation in a commercially available plasmid containing the FLP gene renders the Flp recombinase even more thermolabile. Buchholz et al. recommend "the use of Cre for applications in mice that require efficient recombination." Submitted herewith is a more recent publication by Buchholz et al. (Nat. Biotech., 16:657-662, 1998) in which their goal as stated on page 657 was to obtain "an improved FLP recombinase that would redress inactivation by temperatures relevant to mammalian systems," in contrast to temperatures relevant to yeast (30°C) and *Drosophila* (25°C) systems in which Flp had been used. Caution is apparently needed if one assumes that recombinase activity in different cellular contexts will be identical because the improved Flp recombinase disclosed by Buchholz et al. (1998) is three- to five-fold better in cultured mammalian cells while it is four- to ten-fold better in *E. coli*.

Finally, the motivation to combine the cited references is stated on page 11 of the Action as "to generate a transgenic mouse useful for *in vivo* genetic manipulation." Again, this merely states the result that applicant has achieved and the resultant combination is not rendered obvious unless the prior art suggests the desirability of the combination. Here, the cited references do not suggest the desirability of the combination and there was no reasonable expectation of success in making the claimed invention.

Claims 1, 12, 15, 20, 24, 43 and 47 were rejected under Section 103(a) as being allegedly unpatentable over Orban et al. (1992) and Wigley et al. (1994). Applicant traverses.

Orban et al. teach the use of the Cre-lox system in transgenic mice. Wigley et al. allegedly teach the "potential" use of the Flp recombinase system in transgenic mice. As discussed above, however, Wigley et al. do not suggest the use of Flp in transgenic mice but in ES cells.

It is alleged on page 13 of the Action, "One would have anticipated a reasonable expectation of success because the analogous Cre-loxP system had already been successfully employed." As discussed above, however, the successful use of the Cre recombinase system in transgenic mice and the existence of the Flp-FRT system only establishes the long-felt need for the present invention but the evidence presented above shows there was no reasonable expectation of success when the present invention was made because of the different levels of recombinase activity for Cre and Flp.

If the Examiner maintains that the pending claims are prima facie obvious, she is encouraged to consider the long-felt need for a transgenic mouse with a functional Flp transgene and the evidence of failure by others to make such a transgenic mouse as secondary factors favoring patentability of the claimed invention. See the attached list of 33 investigators who have requested and received Flp-transgenic mice from applicant as evidence of this long-felt need and the failure of others.

For all of the foregoing reasons, applicant respectfully requests withdrawal of the rejections under Section 103.

Having responded to all objections and rejections contained in the pending Office Action, applicants submit that the pending claims are allowable and an early Notice to that effect is earnestly solicited. If further information is needed, the Examiner is invited to contact the undersigned.

Respectfully submitted,

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Enclosed are copies of the following:

1. A list of 33 investigators who have requested and received a Flp-transgenic mouse from applicant.
2. Buchholz et al. (1998) Nat. Biotech., 16:657-662.
3. Dymecki and Tomasiewicz (1998) Dev. Biol., 201:57-65.
4. Meyers et al. (1998) Nat. Genet., 18:136-141.
5. Minichiello et al. (1998) Neuron, 21:335-345.
6. O'Gorman and Wahl (1997) Science, 277:1025.
7. Sauer (1994) Curr. Opin. Biotech., 5:521-527.
8. Vooijs et al. (1998) Oncogene, 17:1-12.

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